Calcium (Arsenazo)

DiaSystem Scandinavia AB

Diagnostic reagent for quantitative in vitro determination of calcium in serum, plasma or urine on photometric systems

Order Information

Cat. No.	Kit size
01 00010 70 04 0125	R: 5 x 25 mL
01 00010 70 04 0500	R: 4 x 100 mL
01 00010 70 10 0160	R: 4 x 40 mL
CDT-Ca	R: 4 x 30mL
01 00010 70 02 0240	R: 4 x 40 mL
06 00101 70 04 0018	Calcium Standard 6x3 mL

Summary [1,2]

Calcium lays 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

Photometric test using arsenazo III

Principle

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

Reagents

Components and Concentrations

Reagent:		
Phosphate buffer	pH 7.5	50 mmol/L
8-Hydroxyquinoline-5-sulfonic acid		5 mmol/L
Arsenazo III		120 µmol/L
Standard		10 mg/dL (2.5 mmol/L)

Storage Instructions and Reagent Stability

Reagent and standard are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and contamination is avoided. Do not freeze the reagent! Protect the standard from light!.

Warnings and Precautions

1. As calcium is an ubiquitous ion, essential precaution must be taken against accidental contamination. Only use disposable materials.
2. Traces of chelating agent, such as EDTA can prevent the formation of the colored complex.
3. The reagent contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
4. In very rare cases, samples of patients with gammopathy might give falsified results [7].
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagent and the standard are ready-to-use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or urine
Do not use EDTA plasma.

Stability [5]

in Serum/Plasma:	7 days	at	20 - 25 °C
	3 weeks	at	4 - 8 °C
	8 months	at	-20 °C
in Urine:	2 days	at	20 - 25 °C
	4 days	at	4 - 8 °C
	3 weeks	at	-20 °C

Add 10 mL of concentrated HCl to 24 h urine and heat the specimen to dissolve calcium oxalate. Discard contaminated specimens. Freeze only once!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	650 nm, Hg 623 nm (630 – 670 nm)
Optical path	1 cm
Temperature	20 – 25 °C / 37 °C
Measurement	against reagent blank

	Blank	Sample or standard
Sample or standard	-	10 µL
Dist. water	10 µL	-
Reagent	1000 µL	1000 µL
Mix, incubate for 5 min and read absorbance against reagent blank.		

Calculation

With standard or calibrator

$$\text{Calcium}[\text{mg/dL}] = \frac{\text{A Sample}}{\text{A Std/Cal}} \times \text{Conc. Std/Cal}[\text{mg/dL}]$$

Conversion factor

Calcium [mg/dL] x 0.2495 = Calcium [mmol/L]
Calcium/U [mg/24 h] x 0.025 = Calcium/U [mmol/24 h]

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Calibrators and Controls

For calibration of automated photometric systems the DiaSystem UniCal CC calibrator is recommended. This method has been standardized against the reference method Atomic Absorption Spectrometry (AAS). For internal quality control DiaSystem UniLab N and DiaSystem UniLab P or DiaSystem UniLab Urine controls should be assayed. Each laboratory should establish corrective actions in case of deviations in control recovery.

	Cat. No.	Kit size
UniCal CC	06 00122 70 04 0018	6 x 3 mL
UniLab N	07 00123 70 05 0030	6 x 5 mL
UniLab P	07 00124 70 05 0030	6 x 5 mL
UniLab Urine Level 1	07 00125 70 04 0030	6 x 5 mL
UniLab Urine Level 2	07 00126 70 04 0030	6 x 5 mL

Performance Characteristics

Measuring range

The test has been developed to determine calcium concentrations within a measuring range from 0.04 – 20 mg/dL (0.01 – 5 mmol/L). When values exceed this range, samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Specificity / Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, hemoglobin up to 500 mg/dL, lipemia up to 2000 mg/dL triglycerides and magnesium up to 15 mg/dL. Strontium salts in medicine may lead to strongly increased calcium values. For further information on interfering substances refer to Young DS [6].

Sensitivity / Limit of Detection

The lower limit of detection is 0.04 mg/dL (0.01 mmol/L).

Precision (at 20-25 °C)

Intra-assay precision	Mean	SD	CV
n = 20	[mg/dL]	[mg/dL]	[%]
Sample 1	8.79	0.09	1.04
Sample 2	12.5	0.15	1.20
Sample 3	14.0	0.24	1.73

Inter-assay precision	Mean	SD	CV
n = 20	[mg/dL]	[mg/dL]	[%]
Sample 1	8.82	0.18	2.01
Sample 2	12.3	0.11	0.90
Sample 3	13.7	0.26	1.92

Method Comparison

A comparison of DiaSystem Calcium (Arsenazo) (y) with a commercially available test (x) using 70 samples gave following results: y = 1.02 x - 0.20; r= 0.999.

Reference Range

Serum / Plasma [2]: 8.6 – 10.3 mg/dL (2.15 – 2.57 mmol/L)

Urine:	Women	< 250 mg/24 h	(6.24 mmol/24 h)
	Men	< 300 mg/24 h	(7.49 mmol/24 h)

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 231–241.
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4. Bauer PJ. Affinity and stoichiometry of calcium binding by arsenazo III. Anal Biochem 1981;110:61-72.
5. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001. p. 20-1 and p. 50-1.
6. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
7. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240–1243.

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