Quantitative determination of Calcium in serum / plasma / Urine
Only for *In Vitro* Diagnostic use

ORDER INFORMATION

REF	Cont.
CARZM 50	50 X 1 mL
CARZ 100	2 X 50 mL

CLINICAL SIGNIFICANCE

Calcium is the most abundant and one of the most important minerals in the human body. Approximately 99% of body calcium is found in bones. A decrease in albumin level causes a decrease in serum calcium. Low levels of calcium are found in hypoparathyroidism, pseudohypoparathyroidism, vitamin D deficiency, malnutrition and intestinal malabsorption. Among causes of hypercalcemia are cancers, large intake of vitamin D, enhanced renal retention, osteoporosis, sarcosidosis, thyrotoxicosis, hyperparathyroidism, multiple myeloma, idiopathic hypercalcemia of infancy, and carcinoma metastasic to bone. Elevated calcium concentration in urine is found in nephrolithiasis and metabolic acidosis.

METHOD

Photometric test using arsenazo III

PRINCIPLE

Calcium with Arsenazo III (1, 8-Dihydroxy-3,6-disulpho-2,7-naphthalene-bis (azo)-dibenzenearsonic acid), at neutral pH, yields a blue colored complex. The intensity of the colour formed is proportional to the calcium concentration in the sample.

REAGENT

Reagent 1 : Arsenazo III Reagent
Calcium Std : 10 mg/dL

REAGENT PREPARATION

All Reagents are ready to use

REAGENT STORAGE AND STABILITY

When stored at recommended storage temperature stated on label, reagent is stable until the expiration date stated on the bottle and kit box label

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.
- Proceed carefully with this product because due to its nature it can get contaminated easily.
- Most of the detergents and water softening products used in the laboratories contain chelating agents. A defective rinsing will invalidate the procedure.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- NaCl solution 9 g/L
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

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

Stability
in Serum/Plasma: 3 weeks at 4 – 8°C
8 months at –20°C
in Urine: 1 day 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

Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned work load.
- Bring all reagents, standard and samples to room temperature 18 - 28ºC, prior to analysis.

AUTOMATED PARAMETERS	
Wavelength	650 nm (620 – 650 nm)
Measurement	Against Reagent blank
Reaction Temperature	RT (22-30ºC)
Reaction Type	End Point
Reaction Direction	Increasing
Incubation	5 Min.
Sample Volume	25 µL
Reagent I Volume	1000 µL
Blank Absorbance Limit	< 0.80
Units	mg/dL

MANUAL ASSAY PROCEDURE

Pipette into Test Tubes

	Blank	Standard	Test
Reagent 1	1000µL	1000µL	1000µL
Standard	--	25µL	--
Sample	--	--	25µL

- Mix Well & Incubate it for 5 minutes at RT (22-30ºC)
- Read and record absorbance of the reagent blank, standard, Control and each unknown sample immediately.
- CARZ 50 are specially treated monovials with 1ml pre-dispensed reagent. Just add 25 µl sample / std., Incubate at R. T. for 5 min. & aspirate. Use the same programme as above.

SAMPLE DILUTIONS

- This method is linear upto a concentration of 16 mg/dL.
- Dilute samples above this concentration 1:1 with DI Water and
- Repeat assay. Multiply the result by 2.

CALCULATION

$$\text{Serum/Plasma} = \frac{(Ac)\ \text{Sample}}{(As)\ \text{Standard}} \times 10\ (\text{Standard concentration.})$$

$$\text{Urine 24Hr.} = \frac{(Ac)\ \text{Sample}}{(As)\ \text{Standard}} \times 10 \times \text{vol. (dL) urine/24 h} \times 2$$

CALIBRATORS AND CONTROLS

For the calibration of automated photometric systems the commercially available suitable multi-calibrator is recommended.

This method has been standardized against the reference method Atomic Absorption Spectrometry (AAS).

It is recommended to run a normal and a pathological control serum which is commercially available to verify the performance of the measured procedure. The value of controls should fall within the established limit.
Each laboratory should establish corrective action in case of deviations in control recovery.

PERFORMANCE CHARACTERISTICS

WITHIN RUN

Sample	Mean Concentration	SD	CV %
Norm	11.25	0.37	3.29%
Path	16.12	0.51	3.20%

RUN TO RUN

Sample	Mean Concentration	SD	CV %
Norm	10.50	0.21	2.01%
Path	16.01	0.44	2.76%

LINEARITY

This method is linear upto a concentration of 16 mg/dL.
Dilute samples above this concentration 1:1 with DI Water and Repeat assay. Multiply the result by 2.

Limit of detection: The limit of detection for Calcium is 0.04 mg/dl.

METHOD COMPARISON

A comparison of Accucare Calcium with a commercially available assay (x) using 59 samples gave following results: $R^2 = 0.9500$

REFERENCE VALUES

Serum:	**8.8 - 10.2 mg/dl**	**= 2.2 - 2.55 mmol/L**
Urine :	**100 - 300 mg/24 h**	**= 2.5 - 7.5 mmol/24 h**

The reference values are to be considered as indicative only. Every laboratory should establish its own normal range.

LIMITATION OF THE PROCEDURE

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

INTERFERENCE

- Bilirubin: No interference found upto 50mg/dl.
- Hemoglobin: : No interference found upto 450 mg/dL.
- Lipemia: Lipids interferences are possible at 660 nm single wavelength, try using bi-chromatic wavelength 660/700 nm to avoid interferences.
- These characteristics have been obtained using an automatic analyzer. Results may vary if a different instrument or a manual procedure is used.

BIBLIOGRAPHY

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GLOSSARY OF SYMBOL

	Consult Instruction for Use	LOT	Lot Number
REF	Catalog Number		Date of Manufacturing
	Store between		Use By or Expiration Date
	Manufacturer	IVD	For *in vitro* Diagnostic use only
	Keep away from sunlight	CONT	Content of the kit

LAB-CARE DIAGNOSTICS (INDIA) PVT. LTD.
C1 Type, Shed No.: 3225, Chemical Zone,
GIDC Sarigam – 396155, Dist. Valsad, Gujarat, India.
Tel.: +91 22 2554 2109 /1558
Email: accucarediagnostics.com; Website: www.labcarediagnostics.com

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