

Diagnostic reagent for the in-vitro quantitative determination of Calcium in human serum, plasma and urine on manual systems.

REF: V/CA02.025	50 test	REF: V/CA02.050	100 test
REF: V/CA04.025	100 test	REF: V/CA02.100	200 test

CLINICAL SIGNIFICANCE

Calcium is the fifth most common element in the body, most of which (98 %) is present in the skeleton. One half of the remaining calcium is found in extracellular fluid and the rest in tissues. Calcium has a crucial role in bone mineralization and is also vital for basic physiological processes such as blood coagulation, neuromuscular conduction, and normal muscle tone. Calcium is constantly lost from the body through excretion in feces, urine and to a small extent in sweat. The determination of serum calcium is useful for monitoring myeloma, renal failure, acid base balance, and cirrhosis. Both serum and tissue calcium in the body are controlled by parathyroid hormone, calcitonin and vitamin D. Hypocalcemia may be observed in hypoparathyroidism, steatorrhea, pancreatitis and nephrosis. Increased levels may be associated with multiple myeloma and other neoplastic diseases.

METHOD PRINCIPLE

Colorimetric. Arsenazo III.

At a neutral pH, with Ca²⁺ form with Arsenazo III a complex, the color intensity of which is directly proportional to the concentration of calcium in the sample

REAGENT COMPOSITION

R1: Standard	10 mg/dl
R2: Reagent MES PH 6.4 Arsenazo III	100 mmol/L 200 µmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment.

For further information, refer to the **Lab.Vie**. Calcium Arsenazo III reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Lab.Vie. Calcium Arsenazo III reagents are supplied ready-to-use. All reagents are stable until expiration date stated on label when stored at 2-8°C.

Deterioration

The **Lab.Vie**. Calcium Arsenazo III reagent is normally clear, do not use reagent if it is turbid.

SPECIMEN COLLECTION AND PRESERVATION

Serum and Plasma

Use nonhemolyzed serum. Heparin is the only acceptable anticoagulant. No other anticoagulant can be used. Fresh serum collected in the fasting state is the preferred specimen. Serum or plasma should be separated from cells as soon as possible, because prolonged contact with the clot may cause lower calcium values. Sera from patients receiving EDTA (treatment of hypercalcemia) are unsuitable for analysis, since EDTA will chelate the calcium and render it unavailable for reaction with O-cresolphthalein complexone. The biological half-life of calcium in blood is few hours.

Urine

Specimens should be collected in acid washed bottles. 24 hour Specimens should be collected in containers containing 5 ml of 6mol/L HCl. If the specimen is collected without acid, the pH should be adjusted < 3 with 6 mol/L HCl. Dilute urine specimen with distilled water (1volume urine + 1volume distilled water) before assay.

Stability (serum): 7 days at 15 – 25 °C; 3 weeks at 4 – 8 °C; 8 months at -20 °C.

Stability (urine): 2 days at 15 – 25 °C; 4 days at 4 – 8 °C; 3 weeks at -20 °C.

Stored serum or urine specimens must be mixed well prior to analysis.

SYSTEM PARAMETERS

Wavelength	650 nm (600 nm)
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample Reagent Ratio	1:100
e.g.: Reagent volume	1 ml
Sample volume	10 µl
Temperature	15-25°C
Incubation time	3 min at 15-25°C
Zero adjustment	Reagent blank
Sensitivity	2.5 mg/dl
Linearity	15 mg/dl

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes, automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

	Blank	Standard	Specimen
Standard		10 µl	
Specimen			10 µl
Reagent	1.0 ml	1.0 ml	1.0 ml

Mix, incubate for 3 minutes at room temp. Measure absorbance of specimen and standard against reagent blank. The color is stable for at least 1 hour.

CALCULATION

Calcium concentration (mg/dl) = $\frac{\text{Abs. specimen}}{\text{Abs. standard}} \times 10$

Urine Calcium (mg/24 hrs.) = $\frac{\text{Abs. specimen}}{\text{Abs. standard}} \times 10 \times 10^* \times 2^{**} \times V^{***}$

* The factor "10" converts mg/dl to mg/liter

** The factor "2" represents the dilution factor

*** "v" represents the 24-hour urine volume in liters

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **Lab.Vie** technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean mg/dl	9.58	13.97	9.6	14.15
SD.	0.12	0.207	0.23	0.221
CV. %	1.33	1.48	1.42	1.53

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)

Result obtained from **Lab.Vie**. Calcium Arsenazo III reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.979.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 2.5 mg/dl.

Linearity

The reaction is linear up to concentration of 15 mg/dl. Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result×2).

INTERFERING SUBSTANCES

Haemoglobin

Avoid haemolysis.

Icterus

No significant interference.

lipemia

No significant interference.

Anticoagulants

Complexing anticoagulants such as citrate, oxalate and EDTA must be avoided.

EXPECTED VALUES











	mg/dl	mmol/l
Serum / Plasma		
Adults 20-50 years	8.8 – 10.2	2.20 – 2.55
Adults > 50 years	8.4 – 9.7	2.09 – 2.42
Children 4-18 years	9.2 – 11.0	2.30 – 2.75
Children >4 years	7.2 – 11.2	1.80 – 2.8
Urine(24 h)	<250 mg/day	<6.25 mmol/day
Females	<300 mg/day	<7.5 mmol/day
Males	<6 mg/Kg/day	<0.15 mmol/day
Children		

DYNAMIC RANGE

2.5– 15 mg/dl.

REFERENCES

1. Barnett RN: A scheme for the comparison of quantitative methods. AM J Clin Pathol 43: 562, 1965.
2. Fiereck EA: Appendix. Normal values. in: Fundamentals of clinical chemistry. NW Tietz, editor, Saunders, Philadelphia, p1208, 1976.
3. Kessler G, wolfsman M: An automated procedure for the simultaneous determination of calcium and phosphorus. Clin Chem 10:686, 1964.
4. Peters JP, Van Slyke, DD: Quantitative clinical chemistry, vol 2, Williams and Wilkins, Baltimore (MD), 1932, p 760.
5. Tietz NW: Blood gases and electrolytes. In: Fundamentals of clinical chemistry, NW Tietz, editor, Saunders, Philadelphia, 176, pp 903, 908.
6. Young DS, Effects of drugs on clinical laboratory tests. AACC press, Washington, D.C. 1990.

SYMBOLS IN PRODUCT LABELLING		
	For in-vitro diagnostic use	 Number of <n> test in the pack
	Batch Code/Lot number	 Caution
	Catalogue Number	 Do not use if package is damaged
	Temperature Limitation	 Consult Instruction for use
	Expiration Date	
	Manufactured by	