COD 11570 1 x 200 mL	COD 11571 1 x 500 mL
STORE AT 2-8ºC	
Reagents for measurement of calcium concentration Only for *in vitro* use in the clinical laboratory	

CALCIUM-ARSENAZO

BioSystems

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CALCIUM-ARSENAZO
ARSENAZO III

PRINCIPLE OF THE METHOD

Calcium in the sample reacts with arsenazo III forming a coloured complex that can be measured by spectrophotometry[1].

CONTENTS

	COD 11570	COD 11571
A. Reagent	1 x 200 mL	1 x 500 mL
S. Standard	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent. Arsenazo III 0.2 mmol/L, imidazole 75 mmol/L.

DANGER: H360: May damage fertility or the unborn child. P201: Obtain special instructions before use. P202: Do not handle until all safety precautions have been read and understood. P280: Wear protective gloves/protective clothing/eye protection/face protection. P308+P313: IF exposed or concerned: Get medical advice/attention. P405: Store locked up.

S. Calcium/Magnesium Standard. Calcium 10 mg/dL (2.5 mmol/L), magnesium 2 mg/dL. Aqueous primary standard.

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE

Store at 2-8ºC.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.550 at 650 nm.

– Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer able to read at 650 ± 20 nm.

SAMPLES

Serum, heparinized plasma or urine collected by standard procedures (Note 1).

Calcium in serum or plasma is stable for 10 days at 2-8ºC. Anticoagulants other than heparin should not be used.

Collect a 24-hour urine specimen in a bottle containing 10 mL of 50 % (v/v) nitric acid. Stable for 10 days at 2-8ºC. Centrifuge or filter and dilute ½ with distilled water before testing.

PROCEDURE

1. Bring the Reagent to room temperature.
2. Pipette into labelled test tubes: (Notes 2,3)

	Blank	Standard	Sample
Calcium Standard (S)	—	15 µL	—
Sample	—	—	15 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

3. Mix thoroughly and let stand the tubes for 2 minutes at room temperature.
4. Read the absorbance (A) of the Standard and the Sample at 650 nm against the Blank. The colour is stable for at least 1 hour.

CALCULATIONS

The calculation of the calcium concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} \times Sample\ \ dilution\ \ factor = C_{Sample}$$

If the Calcium Standard provided has been used to calibrate (Note 4):

	Serum and plasma	Urine
$\frac{A_{Sample}}{A_{Standard}}$	x 10 = mg/dL calcium x 2.5 = mmol/L calcium	x 20 = mg/dL calcium x 5 = mmol/L calcium

REFERENCE VALUES

Serum and plasma[2]: 8.6-10.3 mg/dL = 2.15-2.58 mmol/L

Urine[2]: 100-300 mg/24-h = 2.5-7.5 mmol/24-h

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 0.2 mg/dL calcium = 0.05 mmol/L calcium.

– Linearity limit: 18 mg/dL calcium = 4.5 mmol/L calcium. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean calcium concentration	CV	n
9.6 mg/dL = 2.40 mmol/L	1.7 %	20
13.5 mg/dL = 3.38 mmol/L	1.2 %	20

– Reproducibility (run to run):

Mean calcium concentration	CV	n
9.6 mg/dL = 2.40 mmol/L	2.2 %	25
13.5 mg/dL = 3.38 mmol/L	2.8 %	25

– Sensitivity: 56 mA·dL/mg = 224 mA·L/mmol

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 3). Details of the comparison experiments are available on request.

– Interferences: Bilirubin (< 20 mg/dL) does not interfere. Hemolysis (hemoglobin 2.5 g/L) and lipemia (10 g/L) interfere. Other drugs and substances may interfere[3].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Calcium is the most prevalent cation found in the body, distributed in bone (99%), soft tissues and extracellular fluid. Its concentration in plasma is regulated by parathyroid hormone, vitamin D and calcitonin.

Calcium ion is important in the transmission of nerve impulses, in the maintenance of normal muscle contractility, as a cofactor in certain enzyme reactions, and in the coagulation of the blood.

Hypercalcemia can be due to vitamin D intoxication, enhanced renal retention, osteoporosis, sarcosidosis, thyrotoxicosis, hyperparathyroidsm, multiple mieloma, idiopathic hypercalcemia of infancy, and carcinoma metastasic to bone[2,4].

Elevated calcium concentration in urine is found in nephrolithiasis and metabolic acidosis[2,4].

Hypocalcemia may be caused by primary and secondary hypoparathyroidism, pseudohypoparathyroidism, vitamin D deficiency, malnutrition and intestinal malabsorption[2,4].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. Some plasma sample recoveries may be higher than that expected with serum.
2. Contamination of glassware with calcium will affect the test. Use acid-washed glassware or plastic tubes.
3. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
4. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY

1. Michaylova V, Illkova P. Photometric determination of micro amounts of calcium with Arsenazo III. Anal Chim Acta 1971; 53:194-198.
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