CALCIUM

Arsenazo III Single Reagent

INTENDED USE

Vitro calcium reagent is intended for the in vitro quantitative determination of calcium in human serum, plasma and urine on both automated and manual systems.

METHOD

Colorimetric endpoint method based on the metallochromogen Arsenazo III

BACKGROUND

Calcium has numerous functions within the body, not only as a structural factor in bones and teeth, but also in normal neuromuscular function and the clotting of blood. The calcium content of an adult is somewhat over 1.0 kg i.e. about 2% of the body weight. Of this, 99% is present as calcium hydroxyapatite in bones and less than 1% is present in the extra-osseous ICS (intracellular space) or ECS (extracellular space). The calcium level in the ECS is in dynamic equilibrium with the rapidly exchangeable fraction of bone calcium. Calcium ions affect the contractility of the heart and the skeletal musculature and are essential for the function of the nervous system. In addition, calcium ions play an important role in blood clotting and bone mineralization. In plasma, calcium is bound to considerable extent to proteins (40%), 10% is in the form of inorganic complexes and 50% is present as free (ionized) calcium. The body's calcium balance is regulated by parathyroid hormone (PTH), calcitriol (CT) and calcitonin. The test is used for the diagnosis and monitoring of hypocalcemia (calcium deficiency) and hypercalcemia (excess calcium) in serum. The characteristic symptoms of hypocalcemia are latent or manifest tetany and osteomalacia. Hypocalcemia is due to the absence or impaired function of the parathyroid or impaired vitamin D-synthesis. Hypercalcemia is brought about by increased mobilization of calcium from the skeletal system (osteoporosis) or increased intestinal absorption. The majority of cases are due to primary hyperparathyroidism (pHPT) or bone metastasis of carcinoma of the breast, prostate or thyroid and bronchial carcinoma. The main significance of determining urinary calcium lies in the differentiation between hypercalciuria and hypocalciuria and the differential diagnosis of nephrolithiasis[2,3].

ASSAY PRINCIPLE

Many colorimetric methods have been developed for the determination of calcium. These methods include colorimetric, fluorescent, gravimetric, ion selective, titrimetric, and atomic absorption techniques. Connerty and Briggs described methods using alizarin 3-sulphonate[4] and cresolphthalein complexone[5] whilst Gindler and King have described a method using thymol blue[6]. There have been many subsequent modifications to these methods.

The method used here is based on the metallochromogen Arsenazo III. Arsenazo III combines with calcium ions at pH 6.75 to form a highly coloured chromophore, the absorbance of which is measured at 650 nm. Arsenazo III has a high affinity (K° = 1x10-7) for calcium ions and shows no interference from other cations normally present in serum, plasma or urine.

The intensity of color measured photometrically between 630 and 660 nm with maximum absorbance at 650 nm is directly proportional to calcium concentration in the specimen.

EXPECTED VALUES

Serum or plasma[7]	
Children (<10 days)	7.6 – 10.4 mg/dl (1.9 – 2.6 mmol/l)
Children (< 2 years)	9.0 – 11.0 mg/dl (2.25-2.75 mmol/l)
Children (2-12 years)	8.8 - 10.8 mg/dl (2.2 – 2.7 mmol/l)
Adults (12-60 years)	8.4 – 10.2 mg/dl (2.1 – 2.55 mmol/l)
Adults (60-90 years)	8.8 – 10.2 mg/dl (2.2 – 2.55 mmol/l)
Adults (> 90 years)	8.2 – 9.6 mg/dl (2.05 - 2.4 mmol/l)
Urine	
Male	< 300 mg/day (< 7.5 mmol/day)
Female	< 250 mg/day (< 6.25 mmol/day)
Children	< 6 mg/kg/day (<0.15 mmol/kg/day)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.

REAGENTS

R_1	Calcium standard	10.0 mg/dl
R_2	Arsenazo III Chromogen	

- **Reagent Preparation & Stability**

All reagents are stable up to the expiry date given on label when stored at 2 – 8 ° C.

SPECIMEN

- Serum, plasma, and Urine.

* The only acceptable anticoagulant is heparin.

Specimen Preparation & Stability

- **Serum or plasma**
- Fresh serum collected in the fasting state is the preferred specimen.
- Serum or plasma should be separated from blood cells as soon as possible, because prolonged contact with the clot may cause lower calcium value[8].
- Calcium in serum is stable for 7 days at room temperature, 10 days refrigerated at 4°C and for one month when frozen[9].
- **Urine**
- Urine specimens should be collected in acid-washed bottles.
- 24 hours specimens should be collected in containers containing 5 ml of 6.0 mol/l HCl
- Calcium in acidified urine specimens is stable if stored at room temperature, refrigerated or frozen[9].
- Stored urine specimen must be mixed well and diluted prior analysis.

PROCEDURE

- **Manual Procedure**

Wavelength	650 nm 630-660 nm
Cuvette	1 cm light path
Temperature	37 °C
Zero adjustment	against reagent blank

	Blank	Standard	Specimen
R_2	1 ml	1 ml	1 ml
Standard	.....	10 µl	.....
Specimen	.....	.....	10 µl

Mix, and Incubate for 3 minutes at 37°C. Measure the absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.
The color is stable for 60 minutes.

- **Automated Procedure**

User defined parameters for different auto analyzers are available upon request.

CALCULATION

Calculate the calcium concentration in serum by using the following formulae:

Serum Calcium Concentration=

$$\frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \times \text{Standard}$$

- **Unit conversion**

mg/dl x 0.25 = mmol/l

Calculate the calcium concentration in Urine by using the following formulae:

Urine Calcium Concentration mg/24 hrs=

$$\frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \times 10 \times 10^{*} \times d$$

Where:

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Article #

(10) calcium standard concentration
(10^{n}) converts mg/dl to mg/l
(d) dilution factor
(V) the 24 hours urine values in liter

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established calcium values may be routinely used for quality control.
Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**

  The only acceptable anticoagulant is heparin. Complexing anticoagulant such as EDTA, citrate, and oxalate must be avoided.

- **Bilirubin:**

  No significant interference from free or conjugated bilirubin up to a level of 60 mg/dl.

- **Haemolysis:**

  No significant interference up to levels higher than 700 mg/dl haemoglobin.

- **Lipemia:**

  No significant interference.

- **Drugs:**

  Young[10] in 1990 has published a comprehensive list of drugs and substances, which may interfere with this assay.

WARNING & PRECAUTION

- Vitro calcium reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Tourniquet use should be avoided or kept to a minimum in collecting blood samples for calcium analysis. Fist clenching should be avoided.
- Reagent contains cyanide. Poison may be fatal if swallowed. *DON'T PIPETTE BY MOUTH.*
- For batch testing, according to requirements, prepare the working solution by mixing equal volumes of R_2 and R_3 in one tube.
- It is recommended that disposable calcium free tubes be used for this procedure. If glassware is used, it must be washed with 10% diluted HCl.

- The reagent and specimen volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control

PERFORMANCE CHARACTERISTICS

Imprecision
Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run		Between Day	
Control	Level I	Level I	Level I	Level II
Number of samples	20	20	20	20
Mean (mg/dl)	9.11	13.7	9.31	14.1
SD (mg/dl)	0.10	0.15	0.2	0.28
CV (%)	1.1	1.07	2.16	1.98

Method Comparison
Mixed serum and plasma samples were assayed in parallel using Arsenazo III reagent and another similar commercially available reagent. The results were compared by least squares regression and the following statistics were obtained.
Number of Sample Pairs 45
Range of Sample Results 5.3-13.8 mg/dl
Mean of reference method results 9.90 mg/dl
Mean of Calcium Arsenazo III results 9.62 mg/dl
Slope 0.989
Intercept 0.16 mg/dl
Correlation Coefficient 0.9624

Sensitivity
The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a path length of 1 cm.

When run as recommended the sensitivity of this assay is 0.2 mg/dl.

Linearity
When run as recommended, the assay is linear up to 16 mg/dl.

Specimens with values above 16 mg/dl should be diluted with 0.9% NaCl solution or distilled water and reassayed. Multiply the result by the dilution factor.

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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
1611	2 x 25 ml
1612	2 x 50 ml
1613	2 x 100 ml