

# CALCIUM

## 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 cresolphthalein complexone reaction<sup>1</sup>

## 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<sup>2,3</sup>.

## 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<sup>4</sup> and cresolphthalein complexone<sup>5</sup> whilst Gindler and King have described a method using thymol blue<sup>6</sup>. There have been many subsequent modifications to these methods. Vitro calcium reagent is based on the cresolphthalein complexone (CPC) method of Moorehead and Briggs<sup>1</sup>. CPC reacts with calcium and magnesium in alkaline solution to form a deeply coloured complex. 8-hydroxyquinoline is incorporated into the reagent to preferentially bind magnesium and prevent interference from this cation. CPC is an acid-base indicator necessitating the use of a strong buffer to stabilize the pH.

Calcium reacts with cresolphthalein complexone to form purple color complex in alkaline medium.

### Calcium+ o-Cresolphthalein Complexone

Alkaline medium

### Calcium- Cresolphthalein Complexone complex

The intensity of color measured photometrically between 540 and 600 nm with maximum absorbance at 575 nm is directly proportional to calcium concentration in the specimen.

## EXPECTED VALUES

### Serum or plasma<sup>7</sup>

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 <sub>1</sub>	Calcium standard	10.0 mg/dl
R <sub>2</sub>	Diethylamine	3.79 %
	Potassium cyanide	0.06 %
R <sub>3</sub>	o-Cresolphthalein complexone	0.35 mmol/l
	8-hydroxyquinoline	7.0 mmol/l
	HCl	50.0 mmol/l

### • Reagent Preparation & Stability

All reagents are stable up to the expiry date given on label when stored at room temperature.

## SPECIMEN

- Serum, plasma, and Urine.

\* The only acceptable anticoagulant is heparin.

### • 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<sup>8</sup>.
- Calcium in serum is stable for 7 days at room temperature, 10 days refrigerated at 4°C and for one month when frozen<sup>9</sup>.

### • 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<sup>9</sup>.
- Stored urine specimen must be mixed well and diluted prior analysis.

## PROCEDURE

### • Manual Procedure

Wavelength	575 nm 540-600 nm
Cuvette	1 cm light path
Temperature	20-25 °C
Zero adjustment	against reagent blank

	Blank	Standard	Specimen
R <sub>2</sub>	0.5 ml	0.5 ml	0.5 ml
R <sub>3</sub>	0.5 ml	0.5 ml	0.5 ml
Mix well, then add			
Standard	.....	10 µl	.....
Specimen	.....	.....	10 µl

Mix, and incubate for 5 minutes at room temperature. Measure the absorbance of specimen (A<sub>specimen</sub>) and standard (A<sub>standard</sub>) 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=

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

### • 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=

## Absorbance of Specimen

$\frac{\text{Absorbance of Standard}}{\text{Absorbance of Standard}} \times 10 \times 10^3 \times d \times V$

### Where:

- (10) calcium standard concentration
- (10<sup>3</sup>) 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<sup>10</sup> 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<sub>2</sub> and R<sub>3</sub> 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.

Control	Within Run		Between Day	
	Level I	Level I	Level I	Level II
Number of samples	40	40	40	40
Mean (mg/dl)	6.78	9.25	7.83	12.5
SD (mg/dl)	0.15	0.194	0.16	0.18
CV (%)	2.2	2.1	2.0	1.6

## Method Comparison

Mixed serum and plasma samples were assayed in parallel using Calcium-CPC 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 CPC 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.

### BIBLIOGRAPHY

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### SYMBOL DECLARATION

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

### ORDERING INFORMATION

REF	SIZE
1091	2 x 35 ml
1092	2 x 50 ml
1093	2 x 100 ml