

# High-Q Calcium-ML

## Arsenazo III Method

### Intended Use:

Kit for the quantitative determination of calcium in human serum and urine.

### Summary and Clinical Significance:

The calcium content of an adult is somewhat over 1 kg (25000 mmol), 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 extraosseous ICS (intracellular space) or ECS (extracellular space). The calcium level in the ECS (approx. 100 mmol) 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 a considerable extent to proteins (approx. 40%), 10% is in the form of inorganic complexes and 50% is present as free (ionized) calcium. The body's calcium balance is calcitonin. The test is used for the diagnosis and monitoring of regulated by the parathyroid hormone (PTH), calcitriol (CT) and hypocalcemia (calcium deficiency) and hypercalcemia (excess calcium) in serum. The characteristic symptom of hypocalcemia is 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.

### Principle:

Calcium with Arsenazo III at neutral pH yields a Blue Coloured Complex, whose intensity is proportional to the Calcium concentration in the sample. Interference by Magnesium is eliminated by addition of 8-hydroxy quinoline-5-sulphonic acid.

### Reagents:

Buffer (50 mmol/l) pH	7.2
8-hydroxy quinoline-5-sulphonic acid	10 mmol/L
Arsenazo III	110 µmol/L
Surfactants and anti oxidants	

### Reagent Storage and Stability:

The Reagent and Standard are stable at R.T. till the expiry date mentioned on the labels if external contamination of calcium is avoided.

### Specimen Collection and Storage:

1. Fresh Unhemolysed Serum is the preferred specimen.
2. Heparinised plasma may also be used.
3. Don't use E.D.T.A. plasma.

### Warnings and Precautions:

1. As calcium is ubiquitous ion essential precautions must be taken against accidental contamination. Use only disposable material (Test Tubes, Micro tips etc.)
2. Traces of chelating agent such as E.D.T.A. can prevent the formation of coloured complex.
3. Contamination of glassware with calcium will adversely affect the test results.
4. While dispensing the reagent in to the tubes if blue coloured complex is formed which is having more colour than the blank before the addition of the sample think that the tubes are contaminated.
5. Contamination free disposable plastic tubes are only recommended to perform the Calcium Assay

### Expected Values:

Serum	adults	8.4 to 11.5 mg/dl
Urine	women	<250 mg/24 hours
	men	<300 mg/24 hours

### Procedure:

### Notes:

1. Calcium Assay is performed in fresh disposable new test tubes
2. End user must use fresh disposable new microtips while pipetting Calcium Reagent.

Take new disposable new test tubes and label them as Blank (B) Standard (S) and Test (T). Pipette the Reagent, Standard and Specimen using the new microtips to avoid contamination.

Reagent	B	S	T
Calcium Reagent	1.0 ml	1.0 ml	1.0 ml
Calcium Standard (Conc. 10 mg/dl)	----	10 µl	----
Specimen	----	----	10 µl

After adding the specimen/standard in to the reagent mix well. Incubate for 3 minutes at R.T. Read absorbance of Standard (S) and Test (T) against Reagent Blank (B) at 630 nm (600-650).

### Calculations:

$$\text{Serum calcium in mg/dl} = \frac{\text{Abs. of Test}}{\text{Abs. of Standard}} \times 10$$

### System Parameters:

Reaction type (mode)	:	End Point
Wavelength	:	630nm (600-650)
Flow cell temperature	:	37°C
Sample volume	:	10 µl
Reagent volume	:	1000 µl
Standard concentration	:	10.0
Units	:	mg / dl
Blank	:	Reagent
Low normal	:	8.4
High normal	:	11.5
Linearity	:	25



# High-Q Calcium-ML

## Arsenazo III Method

### Limitation interference:

No significant interference was observed from Bilirubin up to 20 mg/dl (Both conjugated and unconjugated Bilirubin) Hemoglobin up to 50 mg/dl, Lipemia as Triglycerides up to 2000 mg/dl, Ascorbic acid up to 50 mg/dl.

### Measuring Range:

Measuring range: 0.2-25 mg/dl. Determine samples having higher concentrations manually dilute with 0.9% NaCl or distilled/deionized water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2). Analytical sensitivity (lower detection limit): Detection limit: 0.2 mg/dl

### Quality Control:

To ensure adequate quality control, the use of commercial control sera is recommended with each assay batch. Use of quality control material checks both, the instrument and reagent functions.

The detection limit represents the lowest measurable calcium concentration that can be distinguished from zero.

### Imprecision:

#### Serum

Reproducibility was determined using controls. The following results were obtained:

Between day			
Sample	Mean mg/dl	SD mg/dl	CV%
Control serum 1	8.17	0.13	1.59
Control serum 2	14.88	0.32	2.15
Control serum 3	29.77	0.58	1.95
Within run			
Sample	Mean mg/dl	SD mg/dl	CV%
Control serum 1	2.21	0.033	1.49
Control serum 2	2.66	0.035	1.32
Control serum 3	3.89	0.031	0.80

### Method comparison:

A comparison of the High-Q Calcium - ML (y) with a commercial obtainable assay (x) gave the following result :  $y = 1.113x - 0.278$ ;  $r = 0.990$

### Notes:

- All glassware and cuvettes should be rinsed with 30% Hydrochloric acid and again rinse with high quality distilled water thoroughly and repeatedly before use.
- If a larger volume of working reagent is required for the absorbance reading, requisite volumes can be taken in multiples keeping the same ratio of reagent to specimen or standard.
- Dilute lipemic samples with normal saline (made in deionised water) and multiply with dilution factor.
- Protect the kit from direct sunlight.
- For accuracy of results, procedure has to be followed meticulously.
- As with all diagnostic procedures the physician should evaluate data obtained by way of this kit in light of other clinical information.

### References:

- Gitelman, H.J. (1967) Annal. Biochem 18, 521.
- Berthelot, E.S. (1973) Clin. Chem Acta 46, 46.



**Pariksha  
Biotech**  
A game changer in IVD
















### Ordering information

Ref./Cat.	Pack Size	Presentation
P-CAL (AZ) - 50	5 x 10 ml	Mono Reagent
P-CAL (AZ) - 100	10 x 10 ml	

## Product Features

- Liquid Stable, Ready to use Mono Reagent.
- One step End Point assay.
- Addition of 8-Hydroxy Quinolene overcomes Magnesium interference
- Results correlate with (O-CPC) and Methyl Thymol Blue Methods.
- Aqueous Calcium standard provided (Standard Conc: 10 mg/dl)
- Linearity: 25 mg/dL

### Symbols used with IVD devices

	Date of manufacture		Manufactured by
	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)		Reagent
	Calibrator Material		Batch code
	Temperature limit		Control
	Consult instructions for use		Keep dry Keep away from rain
	Catalog Number		



Manufactured in India by :  
Pariksha Biotech Pvt Ltd,  
Plot no.1/B-14, SVICE,  
Balanagar,  
Hyderabad-500037  
Telangana State

