



# CALCIUM

(Arsenazo III Method)  
Liquid Reagent

## INTENDED USE:

This reagent kit is used for *in-vitro* quantitative determination of Calcium in human serum .

## TEST PRINCIPLE:

Calcium reacts with Arsenazo III under neutral conditions to form a purple coloured complex, which has maximum absorbance at 650 nm. The intensity of the colour formed is directly proportional to calcium concentration in the sample.

## KIT CONTENTS:

**Reagent 1** : Calcium Reagent

**Reagent 2** : Calcium Standard (10 mg/dl)

**Product Insert** : 01 No.

## PREPARATION OF THE WORKING REAGENT:

All the reagents are ready to use.

## STORAGE AND STABILITY:

Calcium Liquid reagents are stable till the expiry date mentioned on the labels when stored at Room Temperature (21-25°C).

The Reagent-1 is Provided pre-dispensed in microcentrifuge tubes.

## SPECIMEN COLLECTION AND STORAGE:

Use fresh and unhemolysed serum.

Remove serum from clot as soon as possible, since red cell can absorb calcium.

Serum calcium is stable for one week at 2-8°C.

## PRECAUTION:

1. Storage conditions as mentioned on the product label.
2. Do not freeze or expose the reagents to higher temperature it may affect the performance of the kit.
3. Before conducting the assay bring all the reagents to room temperature.
4. Avoid contamination of the reagents during assay process.
5. Use clean glassware free from dust or debris.
6. Glass test tubes often are coated with residues containing calcium, hence glass should be acid washed or plastic test tubes should be used.

## PROCEDURE (Automated):

Refer to specific instrument application Instruction.

## TEST PROCEDURE (Manual):

Pipette into Pre-dispensed reagent 1	Blank	Standard	Test
Reagent 1	Pre-dispensed	Pre-dispensed	Pre-dispensed
Standard	—	10µl	—
Sample	—	—	10µl

Mix and incubate for 1 min at Room temperature (21-25°C) Zero the spectrophotometer and read the absorbances of (A<sub>T</sub>), Standard (A<sub>S</sub>) and Blank (A<sub>B</sub>).

## PROCEDURAL NOTES:

1. Samples with calcium levels above 16 mg/dl should be diluted 1:2 with saline, reassayed and the result multiplied by two.
2. Severely Lipemic samples require a serum blank. Add 10 UL of sample to 1 ml distilled water. Read against water at 650 nm and subtract the absorbance reading from the absorbance of test. Use fresh plastic tubes or glass test tubes rinsed with 1N HCL.
3. The sample size can be increased, in parallel with the standard, upto 25 ul, without any change in performance.

## CALCULATIONS:

$$\text{Calcium (mg/dl)} = \frac{A_T - A_B}{A_S - A_B} \times 10 \text{ (Concentration of Standard)}$$

It is recommended, that the user determine calibration frequency as this will depend on the instrument & type/number of the assays being run.

## EXPECTED VALUES\*:

Serum 8.8 - 10.2 mg/dl

\*It is recommended that each laboratory should establish its own normal range representing its patient population.

## PERFORMANCE:

1. **Linearity:** 16 mg/dl
2. **Comparison:**  $r = 0.99$   
 $y = 0.98x + 0.2$
3. **Precision:**

	Within Run			Run to Run		
	Mean	S. D.	C. V. %	Mean	S. D.	C. V. %
Low	10.0	0.2	1.5	11.0	0.2	1.0
High	14.0	0.2	1.0	14.0	0.2	1.5

#### 4. Specificity:

1. Substances that contain or form a complex with calcium may produce inaccurate results.
2. Bilirubin upto 20 mg/dl and hemoglobin upto 500 mg/dl do not interfere.

#### CLINICAL SIGNIFICANCE:

Increased serum calcium may be observed in hyperparathyroidism, Vitamin D intoxication, multiple myeloma and some neoplastic diseases of the bone. Decreased serum calcium may be observed in hypoparathyroidism, Vitamin D deficiency, nephrosis and nephritis.

#### AUTOMATED APPLICATIONS:

Calcium Liquid reagents can be used with Hitachi 700 series, RA 50, 1000, XT, Express 550, Synchron CX4, LISA 200, BTR 810/820/830, Erbachem-5 etc. Application sheets for use on specific semi automatic, batch and auto analyzers are available on request.

Input parameters for semi auto/auto analysers are given below.

INPUT PARAMETERS	VALUES
Type of reaction	End Point
Wavelength	650 nm
Incubation time	60 sec.
Standard concentration	10 mg/dl
Temperature	CRT (21-25°C)
Upper normal value	10.2 mg/dl
Lower normal value	8.8 mg/dl
Linearity	16 mg/dl
Reagent volume	1.0 ml
Sample/ Standard volume	10 µl

#### QUALITY CONTROL:

For accuracy, it is necessary to run known serum controls with each assay.

#### REFERENCES:

— Baver. et. al., (1981) Clin. Chem. 110 : 61