CALCIUM

Arsenazo III Method

Intended Use:
Calcium Test reagent/kit is a medical device intended for estimation of Calcium in serum/plasma.

Clinical Significance
Calcium, in the body, is found mainly in the bones (approximately 99%). In serum calcium exists equally in a free ionized form and in a bound form (with albumin). Hence decrease in Albumin causes lower Calcium levels and vice versa. Levels of calcium depend on the parathyroid hormone. Increased calcium levels are found in bone tumors', hyperparathyroidism.Decreased levels are found in hypoparathyroidism, renal failure, rickets, Vitamin D deficiency and pancreatitis.

Principle

Calcium+Arsenazo III ⟶ Blue Purple Coloured Complex

Reagent Composition

R1 Calcium Reagent	8 Hydroxyquinoline - 100 mmol/L Arsenazo III - 200 mmol/L Surfactants
Standard	10mg/dl

Working Reagent Preparation
Reagent is ready to use.

Stability and Storage
Reagents are stable at room temp/ below 30°C till the expiry mentioned on the labels.
All the kit contents are stable until the expiry date stated on thelabel. Do not use reagents beyond the expiration date.
Store the vials tightly closed protected from light and preventcontaminations during the use.

Discard if signs of deterioration appear:
- Presence of particles and turbidity.

Materials required
- Photometer or spectrophotometer with a thermostat cell compartment set at 25/30/37ºC, capable of reading at 630 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm path length
- Pipettes to measure reagent and samples.

Sample and Stability
Serum or plasma
Serum or heparinized plasma free of hemolysis.
Calciumis reported to be stable in serum for 7 days at 2-8ºC.

Known Interfering Substances
- Lipemia (intralipid20 g/L) does not interfere.
- Bilirubin (20 mg/dL) does not interfere.
- Hemoglobin (>2 g/L) may affect the results.

Assay Procedure
Pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T)

Addition Sequence	BLANK	STD	TEST
R1 Calcium Reagent	1000µl	1000µl	1000µl
Standard (S)	-	10µl	-
Sample	-	-	10µl

Mix well and incubate at R.T. (<30^{0}C) for 3 min. Measure the absorbance of the Standard (Abs.S), and Test Sample (Abs.T) against Blank at 630 nm.

Calculation

$$\text{Calcium in mg/dl} = \frac{\text{Abs. T}}{\text{Abs.S}} \times 10$$

Quality Control
To ensure adequate quality control (QC), each run should include aset of controls (normal and abnormal) with assayed values handledas unknowns.

If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable results.

Linearity
This procedure is linear upto 20 mg/dl. If values exceed this limit dilute the sample with distilled water and repeat the assay. Calculate the value using proper dilution factor.

Reference Values

Serum / Plasma	: 8.7 – 11.0 mg/dl
Urine	: 100-400 mg/24 hours

It is recommended that each laboratory establish it own normal range representing its patient population.

General System Parameters

Mode	End Point
Reaction	Increasing
Wavelength	630 nm
Blank with	Reagent
Sample Volume	10µl
Reagent Volume	1000µl
Std Conc.	10mg/dl
Incub. Temp.	RT (Below 30°C)
Incub. Time	3 min.
Delay Time	5 sec
Normal Range	8.7 – 11.0 mg/dl
Linearity	Up to 20 mg/dl
Unit	mg/dl

Notes
As calcium is a very widely distributed ion, care should be taken to avoid any contamination. All glassware being used for the test should first be rinsed with 1% or 0.1 N Hcl and then with good quality deionised water before use. For flow cell/cuvettes it is suggested that some reagent be aspirated before the blank to take away any contamination in the flow through tubing or cuvette which may cause a higher than the actual blank of the reagent.
Chelating agents such as EDTA, present even in traces, prevent the formation of the colourcomplex, hence necessary care should be taken during the assay.

Reference
1. Smith, H.G. Jr.et al, (1979) Biochem 18:5067.

For *in vitro* Diagnostic use only.