

CALCIUM ARSENAZO

Arsenazo Method



CLINICAL SIGNIFICANCE :

Calcium exists in three states in biological specimen as free or ionized calcium, calcium complexed to anions (including bicarbonate, lactate, phosphate, and citrate) and calcium bound to plasma proteins. Calcium has key roles in muscle contraction, hormone secretion, glycogen metabolism, and cell division. Increased calcium levels in serum are reported in hyperparathyroidism, cancer, metastatic bone lesions and hypervitaminosis, while decreased levels are observed in hypoparathyroidism, nephrosis, rickets, nephritis and calcium losing syndromes.

TEST PRINCIPLE :



At a neutral pH, the Ca^{++} form with Arsenazo III a complex, the color intensity of which is directly proportional to the concentration of calcium in the sample.

REAGENTS COMPOSITION :

Reagent-1 (R1) : MES pH 6.50, 100 mmol/L
Arsenazo III 200 $\mu\text{mol/L}$

Calcium Standard : Concentration See on Vial

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8 °C, and contaminations prevented during their use. Do not use reagents over the expiration date. Once opened the reagent is stable for 1 month on-board the analyzer at approximately 10°C. .

KIT CONTENTS :

CODE No.	CA01	CA02	CA03
Pack size :	(5x10 ml)	(5x25 ml)	(25x1 ml) MONOTEST
Reagent-1 (R1)			
Arsenazo Reagent	5 x 10 ml	5 x 25 ml	25 x 1 ml
Calcium Standard (Conc. : 9.0 mg/dl)	1 x 1 ml	1 x 1 ml	1 x 1 ml

SAMPLES :

Serum or heparinised Plasma
24 hours-Collected Urine*

Serum should be separated from blood as soon as possible.

*24 hrs-collected Urine: Acidify to pH 3 – 4 with N/10 HCl to dissolve Ca-Oxalate which may be present

ASSAY CONDITIONS:

Wavelength :	620 nm (600 - 650 nm)
Cuvette:	1 cm light path
Constant temperature	37°C
Reaction	End Point
Standard Conc.....	9.0
Unit.....	mg/dl
Linearity.....	20 mg/dl
Unit.....	mg/dl
Slope of Reaction	Increasing
Blanking.....	Reagent

PROCEDURE :

For MONOTEST vial, no need to pipette out the reagent.
Take 3 vials of MONOTEST Reagent and labeled on the top as Blank (B), Standard (S) and Test (T).

Pipette into test tubes labeled Blank (B), Standard (S) and Test (T) as follows **for other pack sizes.**

	B	S	T
Arsenazo Reagent (R1)	1.0 ml	1.0 ml	1.0 ml
Calcium Standard		10 l	
Specimen			10 l

Mix and incubate for 5 mint at R.T. Read absorbance of Standard (S) and Test (T) against Blank (B) with 620 nm. The final color is stable for 1 hour at R.T.

CALCULATIONS :

$$\text{Calcium in mg/dl} = \frac{\text{Abs. of T}}{\text{Abs. of S}} \times 9$$

REFERENCE RANGE :

Serum, plasma : 8.6 - 10.3 mg/dL

Urine : Women < 250 mg/24 h

Men < 300 mg/24 h

The above reference range is guideline and all the laboratories must establish their own normal reference range. Final diagnosis should be made with correlation of clinical factors.

PRECAUTIONS :

- Storage conditions as mentioned on the kit to be adhered.
- For testing use disposable Plastic test tube. Use fresh micropipette tips while pipetting Arsenazo Reagent and Calcium Standard. Replug Reagent and Calcium Standard vial after immediate use.**
- Avoid contamination of the reagent during the assay process.
- Before the assay begins, bring all the reagents to room temp.
- If a larger volume of reagent is required for the absorbance reading, requisite volume can be taken in multiples, keeping the same ratio of reagent to specimen/standard.
- Do not freeze or expose the reagents to high temperature and protect from direct sunlight as it will affect the performance of the kit.
- Programmes for specific autoanalysers are available on request.
- For accuracy of results, the assay procedure, reagent preparation and storage has to be meticulously followed.
- As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.

LINEARITY AND DETECTION LIMIT :

The assay is linear up to Calcium concentration of 20 mg/dl. The results of the performance characteristics depend on the analyzer used. If the results obtained were greater than linearity limit, dilute the sample 1 : 2 with Normal Saline and multiply the result by 2.

BIBLIOGRAPHY

- Tietz, N.W., Fundamentals of Clinical Chemistry 2nd. Edition W.B. Saunders Co., Philadelphia (1976)
- V. Michaylova and P. Ilkova, Photometric determination of micro amounts of calcium with arsenazo III, (1971)Anal. Chim. Acta. 53:194-198
- Barnett, R.N., et al. Performance of "kits" used for clinical chemical analysis of calcium in serum. (1973) Amer. J. Clin. Path. 59: 836-845