CALCIUM (Arsenazo)

(Arsenazo III)

CHEMPAK

Last update 09-2020

Ref. CC1-CAC.37M, 25 Test
CC1-CAC.37MU, 50 Test
CC3-CAC.37MV, 2x60 ml

INTENDED USE

Reagent kit for quantitative estimation of Calcium (Ca^{++}) in serum, plasma.

INTRODUCTION

Earlier calcium estimations were carried out mainly by titrimetric method of Pollard and Schwartzenbach. As magnesium is the primary source of interference in this method of calcium estimation, the reagent has to be modified so as to make it specific for calcium. Many colorimetric method for determining Calcium have been used in the past. Connerty and Briggs described methods using alizarin 3-sulphonate and cresolphthalein complexone whilst Ginder and King have described a method using thymol blue. There have been many subsequent modifications to this methods.

Calcium is a ARSENAZO procedure employing metallo Chromogen Arsenazo as a colour developing agent.

DIAGNOSTIC SIGNIFICANCE

Calcium functions as an important factor in structure of bones and teeth, in neuromuscular activity and in clotting of blood. Elevated calcium values are associated with multiple myeloma, neoplasia of bone., hyperparathyroidism and conditions of rapid demineralization of bone. Lower calcium levels are associated with hypoparathyroidism, tetany and occasionally, with nephrosis or pancreatitis.

PRINCIPLE

Ca^{+2} + Arsenazo III ⟶ Coloured complex

The method used here is based on the metallochromogen Arsenazo III. Arsenazo III combines with calcium ions at pH 6.75 to form a highly coloured chromophore, the absorbance of which is measured at 630 nm. Arsenazo III has a high affinity ($K^0 = 1x10^{-7}$) for calcium ions and shows no interference from other cations normally present in serum, plasma.

PRESENTATION

All reagents to be stored at 2-8^{0}C	No. of Bottles/Vials		
	25 Test	50 Test	2x60 ml
• Calcium Reagent (Ready to use)	25	50	2
• Calcium Standard (10mg/dl)	1	2	2

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• Buffer	80 mmol/L
• Detergent	10 mmol/L
• 8 Hydroxy quinolene	2 mmol/L
• Arsenazo-III	≥ 0.05 mmol/L

pH 6.5 ± 0.1 at 25^0 C
Calcium Standard (10 mg/dl)
Also contains non-reactive fillers and Stabilizers.

PRECAUTION

In case of glasswares to be used in calcium assay they should be thoroughly decontaminated by soaking into 1N HCl. Rinse the glasswares thoroughly with distilled water prior to use.

REAGENT STORAGE AND STABILITY

Calcium reagents are stable at 2-8^{0}C till the expiry date stated on the label.

SPECIMEN COLLECTION

Fresh, clear, fasting serum without hemolysis is necessary.

REACTION PARAMETERS

- Type of Reaction : End Point / Increasing
- Wavelength : 630/650 nm
- Flowcell Temperature : 30^{0}C
- Incubation : 5 min. at RT
- Std. Concentration : 10 mg/dl
- Sample Volume : 10 µl (0.01 ml)
- Reagent Volume : 1.0 ml
- Zero setting with : Reagent Blank
- Light Path : 1.0 cm

TEST PROCEDURE

For instrument having 1.0 ml. cuvette capacity.

Pipette into Test Tubes	BLANK	STANDARD	TEST
Reagent (ml)	1.0	1.0	1.0
Sample (ml)	-	-	0.01
Standard (ml)	-	0.01	-

Mix well and incubate for 5 minutes at room temperature (25-30^{o}C) and read absorbance of standard and sample against reagent blank at 630 nm (630 to 660 nm or with RED filter).

STABILITY OF FINAL REACTION MIXTURE

The colour of final reaction mixture is stable for one hour (when protected from light).

TEST RESULTS

$$\text{Calcium concentration (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Std.}} \times 10$$

$$\text{Conversion Factor mmol/L} = \frac{\text{mg/dl}}{4}$$

NORMAL VALUE

8.5 to 10.5 mg/dl

LINEARITY

This method is linear upto 20 mg/dl. For sample values, higher than 20 mg/dl, dilute the sample suitably with 0.9% saline and repeat the assay. Apply dilution factor to obtain the test results.

NOTE

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. The temperature of the react ion is not critical. however, the temperature of the spectrophotometer should be held constant as the absorbance of the dye is temperature sensitive.
3. Because of the need to obtain highly accurate calcium results, it is recommended that the manual assay should be performed in duplicate.

REFERENCES

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