

CALCIUM

(OCPC METHOD)

Reagent kit for quantitative estimation of calcium (Ca⁺⁺) in serum.

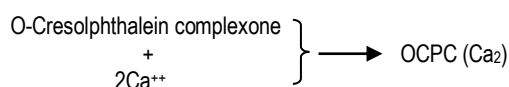
BACKGROUND AND SYNOPSIS:

Earlier calcium estimations were carried out mainly by titrimetric method of Pollard and Schwanzenbach. The primary source of interference with colorimetric methods for calcium has been magnesium, which similarly reacts to some extent with the same compound therefore the reagent has to be modified so as to make it specific for calcium.

CHEMPAK calcium is a modification of Coonerty and Briggs procedure employing OCPC as a colour developing agent and 8-hydroxyquinoline to mask the presence of magnesium. The modified reagent is safe to handle as it is free from potassium cyanide.

PRINCIPLE:

Ortho-cresolphthalein complexone reacts with calcium at pH 10.0 to form a purple coloured complex. The presence of 8-hydroxyquinoline in the reagent prevents interference by magnesium. This method does not require pretreatment of serum sample.



The purple complex formed is measured at 570 nm (530-580 nm or with GREEN filter). The intensity of the colour developed is proportional to the concentration of calcium present in the sample.

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.

Active Ingredients	Concentration
Reagent-1	
* OCPC	0.80 mmol/L
* 8 Hydroxy quinolone	2 mmol/L
Reagent-2	
*Buffer	100 mmol/L
pH 10.5± 0.1 at 25° C	
Calcium Standard (10 mg/dl)	
Also contains non-reactive fillers and Stabilizers.	

PRESENTATION:

	No. of Bottles
All reagents to be stored at 2-8°C.	100 ml
• 1 CALCIUM (COLOUR REAGENT)	1
• 2 CALCIUM (COLOUR DEVELOPING REAGENT)	1
• CALCIUM STANDARD (10 mg/dL)	1

PRECAUTION:

- In case of glasswares to be used in calcium assay they should be thoroughly decontaminated by soaking into 1N HCl overnight (8 to 10 hours) or alternatively, into 6N HCl for 4 to 8 hrs.
Reagent contains Sodium Azide. DO NOT INGEST.

REAGENT PREPARATION:

Prepare the reagent as per the daily requirements of laboratory by mixing equal volumes of 1 CALCIUM and 2 CALCIUM.

REAGENT STORAGE AND STABILITY:

CHEMPAK Calcium reagents are stable at 2-8°C till the expiry date stated on the label. The reagent solution should be clear. Cloudiness or turbidity implies that there is contamination and such reagents should be discarded.

SPECIMEN COLLECTION:

Fresh, clear, fasting serum without hemolysis is necessary.

REACTION PARAMETERS

- | | |
|------------------------|------------------|
| : | : |
| • Type of Reaction | : End Point |
| • Wavelength | : 570 nm |
| • Flowcell Temperature | : 30 °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. |

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	-

For instrument having 2.5 ml. cuvette capacity.

PIPETTE INTO TEST TUBES	BLANK	STANDARD	TEST
• REAGENT (ml)	2.5	2.5	2.5
• SAMPLE (ml)	-	-	0.02
• STANDARD (ml)	-	0.02	-

Mix well and incubate for 5 minutes at room temperature (25-30°C) and read absorbance of standard and sample against reagent blank at 570 nm (530 to 580 nm or with GREEN filter).

STABILITY OF FINAL REACTION MIXTURE:

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

TEST RESULTS:

$$\text{Calcium concentration (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 10$$

NORMAL VALUES:

Serum calcium: 8.7 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.



NOTES:

- (1) For severely icteric, hemolytic or lipemic samples the use of sample blank is recommended which may be prepared by adding 0.01 ml. serum to 1 ml. of distilled water (or 0.02 ml. serum to 2.5 ml. of distilled water as per cuvette capacity).

Alternatively, a sample blank may be prepared by treating the icteric sample with EGTA reagent (procedure and reagent on request). Finally, a corrected sample absorbance is obtained by subtracting sample blank absorbance from absorbance of test sample.

Corrected sample absorbance = Absorbance of test - Absorbance of blank.

- (2) Reagent blank absorbance is generally more than 0.100 but blank absorbance values exceeding 0.300 are not acceptable. This indicates that, there is some contamination either in reagent or in glasswares used or deterioration of reagent.

REFERENCES:

- Schwitzenbach, G. Complexones and Their Analytical Applications, Analyst 80, 348-353, (1956)
- Zak, B., Epstein, E., Baginski, E.S., Review of Calcium Methodologies, Annals of Clinical and Laboratory Science 5, 195-215, (1975)

