CALCIUM KIT

(OCPC method)

For the determination of Calcium in serum or plasma.
(For Invitro Diagnostic Use Only)

Summary

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

Principle

Calcium in an alkaline medium combines with o - Cresolphthalein Complexone to form a purple coloured complex. Intensity of the colour formed is directly proportional to the amount of calcium present in the sample.

Calcium + OCPC ⟶ Purple coloured complex

Normal reference values

Serum / Plasma : 8.7 - 11.0 mg/dl

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

Contents	**2 x 35 ml**	**2 x 75 ml**	**2 x 150 ml**
L1 : Buffer Reagent	35 ml	75 ml	150 ml
L2 : Colour Reagent	35 ml	75 ml	150 ml
S : Calcium Standard (10 mg/dl)	5 ml	5 ml	5 ml

Storage / stability

Contents are stable at 2 - 8 ^{0}C till the expiry mentioned on the labels.

Reagent Preparation

Reagents are ready to use. Protect from bright light.

Working Reagent : For convenience a single working reagent may be prepared by mixing equal parts of the Buffer Reagent and Colour Reagent. This combined reagent is stable for 7 days at 2 - 8 ^{0}C.

Sample material

Serum / Heparinized plasma. Calcium is reported to be stable in serum for 7 days at 2-8^{0}C.

Procedure

Wavelength / filter : 570 nm (Hg 578 nm) / Yellow
Temperature : R.T.
Light path : 1 cm

Pipette into clean dry test tubes labelled as Blank (B), Standard (S), and Test (T):

Addition Sequence	**B** (ml)	**S** (ml)	**T** (ml)
Buffer Reagent (L1)	0.5	0.5	0.5
Colour Reagent (L2)	0.5	0.5	0.5
Distilled water	0.02	-	-
Calcium Standard (S)	-	0.02	-
Sample	-	-	0.02

Mix well and incubate at R.T. (25^{0}C) for 5 min. Measure the absorbance of the Standard (Abs.S), and Test Sample (Abs.T) against the Blank, within 60 Min.

Calculations

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

Linearity

This procedure is linear upto 18 mg/dl. If values exceed this limit, dilute the sample with distilled water and repeat the assay. Calculate the value using the proper dilution factor.

Note

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.

It is suggested that after the rinsing of the tubes with HCl the reagent be pipetted in their respective tubes and the tubes be rinsed with the reagent. The reagent then should be pooled together in the 'blank' tube and repipetted out into the 'standard' and 'test' test tubes. This will ensure that any remaining contamination will be carried over equally in all the tubes. 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 colour complex, hence necessary care should be taken during the assay.

References

Gitelman, H.J., (1967), Anal. Biochem. 18 : 521
Bagainski, E.S., (1973), Clin. Chem. Acta. 46 : 46

System Parameters

Reaction	:	End Point
Wavelength	:	570 nm
Zero Setting	:	Reagent Blank
Incub. Temp.	:	R.T.
Incub. Time	:	5 min.
Delay Time	:	---
Read Time	:	---
No. of read.	:	---
Interval	:	---
Sample Vol.	:	0.02 ml
Reagent Vol.	:	1.00 ml
Standard	:	10 mg/dl
Factor	:	---
React. Slope	:	Increasing
Linearity	:	18 mg/dl
Units	:	mg/dl

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