

Reagent kit for determination of calcium concentration in serum and urine. A colorimetric method based on complex formation with ortho-cresolphthalein.

In the human body 98 - 99% of calcium is present in bound form in bones and teeth. About 50% of the blood calcium circulates in ionic form, the other part as bound to proteins. The concentration of ionic calcium is influenced by the acid-base household of the body. The ratio of ionic/protein-bound calcium is higher in acidosis and lower in alkalosis. Elevated calcium levels are found in association with primary hyperparathyroidism, neoplastic diseases (eg. breast cancer, bronchial cancer, pancreatic tumor), osteoporosis, Paget's disease and Addison's disease, overdosage of the vitamins A and D, hyperthyroidism. Lower calcium values are measured in hypoparathyroidism, disturbances of the absorption, chronic renal failure, nephrotic syndrome, hepatic cirrhosis, acute pancreatitis.

Principle

Calcium in alkaline medium forms a purple-red complex with ortho-cresolphthalein. Intensity of the developed color is proportional to the calcium concentration in the sample.

Reference values

Serum:	newborns	2.3-2.5 mmol/l (9,2-10,0 mg/dl)
	children	2.5-3.0 mmol/l (10,0-12,0 mg/dl)
	adults	2.25-2.75 mmol/l (9,0-11,0 mg/dl)
Urine:		2.5- 8.0 mmol/24 h
		10,0-32,0 mg/24h

It is recommended that each laboratory should assign its own normal range.

Reagents

1. Reagent (R1)	
AMP (2-amino-2-methyl-1-propanol)	400 mmol/l
2. Reagent (R2)	
o-Cresolphthalein	0.62 mmol/l
8-Hydroxyquinoline	69 mmol/l

2. Calcium standard

Ready for use. For details please check the insert.
Available only in Cat. No.: 45241S

Sample

Serum.
24-h collected urine sample diluted with distilled water (1:3). (The pH-value of the urine should be adjusted to pH 3-4 with diluted HCl).

PROCEDURE

Preparation of working reagent

Mix 1 volume part of buffer solution (R1) with 1 volume part of chromogen reagent (R2).

Stability of working reagent

20-25°C	24 hours
2-8°C	7 days

Store protected from light!

If the optical density of working reagent is higher than 0.5 at 570 nm the reagent can not be used.

Assay conditions

Wavelength:	570 (550-590) nm
Temperature:	37 °C
Cuvette:	1 cm light path
Method:	end point (increasing)

Pipette into cuvette

	blank	standard	sample
working reagent	1 ml	1 ml	1 ml
standard		20 µl	
sample			20 µl
distilled water	20 µl		

Mix and incubate the reaction mixtures at room temperature for 5 minutes. Read the optical density against the blank.

Reagents can also be applied without previous mixing. In this case under maintaining the rations, 0.5 ml (R1) and 0.5 ml (R2) should be given to the samples.

Calibration (37°C, cresolphthalein-complexone method)

S1: distilled water
S2: Calcium standard Cat. No.: 52001 or
Roche C.F.A.S. (Calibrator for automated system)
Randox Calibration Serum Level I

S3: Randox Calibration Serum Level II

Calibration frequency

Calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

Calculation using calibration

$$\frac{A_{sample}}{A_{standard}} \times C_{standard} = C_{sample}$$

C=concentration A=absorbance

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits

PERFORMANCES DATA

The following data were obtained using the Olympus 600 analyzer (37°C).

Linearity

The test is linear up to 4.5 mmol/l (18,0 mg/dl) calcium concentration.

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs is equivalent to 0.006 mmol/l (0,024 mg/dl) calcium concentration at 570 nm.

Precision

	Reproducibility		
	Average concentration (mmol/l)	SD	CV%
sample I	2.16	0.046	2.14
sample II	3.62	0.089	2.46

Correlation

Comparative studies were done to compare our reagent with another commercial calcium assay on human serum samples.

The results from these studies are detailed below.

Correlation coefficient: $r=0.9805$
Linear regression: $y \text{ (mmol/l)} = 1.034x - 0.17$
(x= other commercial reagent, y= own reagent).

Note

Trace amounts of EDTA and other chelating agents interfere with the test. Utmost care should be given to purity and freedom from calcium of laboratory equipments and materials used for the tests.

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For in vitro diagnostic use only.

The following symbols are used on labels

-  For in vitro diagnostic use
-  Use by (last day of the month)
-  Temperature limitation
-  Batch Code
-  Code

Bibliography

Stem J., Lewis W.P.: Clin. Chem. Acta 2, 576 (1957)